24. The process as recited in claim 10 wherein the genetic material is bound to chromophore in anaerobic conditions.

25. The process as recited in claim 8 wherein the two buffers comprise a first buffer to lyse the cells and a second buffer to attach the genetic material to the column.

REMARKS

Claims 1, 3, 4, 8, 11 and 13-14 are rejected under 35 U.S.C. §103(a) as being unpatentable over Henco et al. (USPN 5,652,141) and in view of Eldadah et al. (Nucleic Acids Research 24(20): 4092-4093). Claims 1 and 8 are rejected under §112 as being indefinite as to the word "manipulation".

Claims 2, 5-7, 9, 10, 12 and 15-19 are objected to but would be allowable if rewritten in independent form incorporating all limitations of the base claims.

Reconsideration of the application is requested in light of the foregoing amendment. Claim 1 has been amended to recite labeling the immobilized genetic material within the column. Support for said recitation is found on page 6, lines 24-27.

Independent claims 1 and 8 have been further amended by replacing "manipulation" with labeling so as to rectify the 112, second paragraph rejection.

Per the Examiner's indication of allowance of objectionable claims, claims 2, 5, 9, 10, and 13 have been rewritten in independent form to include all limitations of base claims and intervening claims.

Claim 19 has been amended to recite a temperature range of between 30 °C and 100 °C. Support for this limitation can be found on page 12, line 11 of the instant specification.

Claims 20-25 have been added merely to keep the subject matter of the objected to claims (discussed in the immediately preceding paragraph) also dependent from claims 1 and 8.

The drawing have been objected to inasmuch as subunits of the drawing were

not described in the text portion of the brief description of the Drawing section of the specification. Amendment to that section is herein provided.

The oath/declaration was considered defective inasmuch as the residence and citizenship of one of the inventors was missing. Enclosed herewith is a replacement declaration.

Applicant's Invention

As now claimed, a salient feature of the inventive method comprises labeling immobilized genetic material while the genetic material remains within a fractionation/reaction column. This feature consolidates and therefore expedites fractionation and labeling protocols to provide a process measured in minutes, rather than hours. This feature is not anticipated or suggested by the art of record.

Eldadah Does Not Teach Labeling Within a Column

Claims 1, 3, 4, 8, 11 and 13-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Henco et al. (USPN. 5,652,141) and in view of Eldadah et al. (Nucleic Acids Research, 24(20):4092-4093. Eldadah apparently was referenced for its use of labels. Applicant submits that the added limitation in claims 1 and 8 of labeling the immobilized genetic material within the column renders the art of record irrelevant.

Eldadah recites a method that isolates genomic DNA with a silica-based resin column. Once the resin-isolated DNA is extracted from the column, optional labeling of DNA can be done to increase the sensitivity of detection via autoradiography.

Specifically, and as recited on page 4093 (Column 1, last paragraph), Eldadah endeavors to increase the detection of DNA by labeling 1 µg of DNA with 20 µl of labeling mixture. Inasmuch as a specific quantity of DNA is required Eldadah's labeling step, such step could only occur *after* the DNA is isolated from the Wizard Minipreps DNA Purification Resin (Promega).

Either alone, or in combination with Henco et al., Eldadah's method does not anticipate or suggest labeling genetic material residing within a fractionation column.

If Eldadah adopts the now-claimed labeling within the resin-column, the resulting formulation would defeat Eldadah's stated objective of increasing the sensitivity of detecting DNA laddering. If a prior art reference is cited that requires some modification in order to meet the claimed invention and such modification destroys the purpose of the invention disclosed in the reference, one of ordinary skill in the art would not find reason to make the proposed modification. In re Gordon 733 F. 2d 900 (Fed. Cir 1984).

In light of the foregoing amendment, Applicant submits that the §103(a) rejection of claims 1, 3, 4, 8, 11 and 13-14 are obviated. Withdrawal of said rejection and allowance of said claims is respectfully solicited.

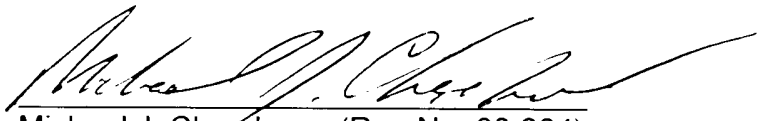
112 Rejection Obviated
With Genetic Manipulation

Claims 1 and 8 are rejected under §112, second paragraph as being indefinite. Applicant submits that in light of the amendment to claim claims 1 and 8, wherein "manipulating" is replaced by labeling the rejection is now moot.

An earnest attempt has been made hereby to respond to the §§103 and 112 rejections contained in the September 24, 2001 Official Action. It is submitted that all remaining claims are of proper form and scope for allowance. If the Examiner feels that a telephonic interview would expedite allowance of this application, he is respectfully urged to contact the undersigned. Reexamination and allowance of claims 1-19 and consideration and allowance of newly added claims 20-25 is respectfully solicited.

Respectfully submitted,

CHERSKOV & FLAYNIK



Michael J. Cherskov (Reg No. 33,664)

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1. (Amended) A method for [manipulating] labeling genetic material, the method comprising:

a) disrupting cells so as to liberate genetic material contained in the cells;

b) contacting the genetic material to a column in a manner to cause the genetic material to become immobilized to the columm;

c) labeling the immobilized genetic material within the column; and

d) eluting the labeled material from the column.

2. (Amended) A method for manipulating genetic material, the method comprising:

a) disrupting cells so as to liberate genetic material contained in the cells;

b) contacting the genetic material to a column in a manner to cause the genetic material to become immobilized to the column;

c) labeling the immobilized genetic material; and

d) eluting the labeled material from the column [The method as recited in 1] wherein the step of labeling the genetic material further comprises maintaining the column at a temperature of between 45 °C and 100 °C.

5. (Amended) A method for manipulating genetic material, the method comprising:

a) disrupting cells so as to liberate genetic material contained in the cells;

b) contacting the genetic material to a column in a manner to cause the genetic material to become immobilized to the column;

c) labeling the immobilized genetic material; and

d) eluting the labeled material from the column [The method as recited in claim 1] wherein the step of labeling the genetic material comprises:

[a)] e contacting double-stranded nucleic acid molecules of the genetic material with radical-generating complexes for a time and at concentrations sufficient to produce free-aldehyde moieties;

[b] f reacting the aldehyde moieties with amine to produce a condensation product; and

[c] g contacting the condensation product with a chromophore.

8. (Amended) A two-buffer process for [manipulating] labeling genetic material, the process comprising:

a) contacting cells containing the genetic material to a silica column;

b) creating a first fraction of cell detritus and a second fraction containing the genetic material;

c) confining the genetic material to the column;
d) removing the cell detritus;
e) subjecting the genetic material to radicals so as to produce reactive aldehyde groups on the genetic material; and
f) attaching chromophore to the genetic material while the material resides in the column.

9. (Amended) A two-buffer process for manipulating genetic material, the process comprising:
a) contacting cells containing the genetic material to a silica column;
b) creating a first fraction of cell detritus and a second fraction containing the genetic material;
c) confining the genetic material to the column;
d) removing the cell detritus;
e) subjecting the genetic material to radicals so as to produce reactive aldehyde groups on the genetic material; and
f) attaching chromophore to the genetic material [The process as recited in claim 8] wherein the genetic material is contacted with radical in aerobic conditions.

10. (Amended) A two-buffer process for manipulating genetic material, the process comprising:
a) contacting cells containing the genetic material to a silica column;
b) creating a first fraction of cell detritus and a second fraction containing the genetic material;
c) confining the genetic material to the column;
d) removing the cell detritus;
e) subjecting the genetic material to radicals so as to produce reactive aldehyde groups on the genetic material; and
f) attaching chromophore to the genetic material [The process as recited in claim 8] wherein the genetic material is contacted with radical in anaerobic conditions.

13. (Amended) A two-buffer process for manipulating genetic material, the process comprising:
a) contacting cells containing the genetic material to a silica column;
b) creating a first fraction of cell detritus and a second fraction containing the genetic material;
c) confining the genetic material to the column;
d) removing the cell detritus;
e) subjecting the genetic material to radicals so as to produce reactive aldehyde groups on the genetic material; and
f) attaching chromophore to the genetic material [The process as recited in

claim 8] wherein the two buffers comprise a first buffer to lyse the cells and a second buffer to attach the genetic material to the column.

19. (Amended) The process as recited in claim 8 wherein the temperature is maintained at [95 °C] between 30 °C and 100 °C.

20. (New) The method as recited in claim 2 wherein the column comprises a means for subjecting the silica to pressure.

21. (New) The method as recited in claim 1 wherein the step of labeling the genetic material comprises:

a) contacting nucleic acid molecules of the genetic material with radical-generating complexes for a time and at concentrations sufficient to produce free-aldehyde moieties;

b) reacting the aldehyde moieties with amine to produce a condensation product; and

c) contacting the condensation product with a chromophore.

22. (New) The method as recited in claim 21 wherein the step of contacting the condensation product with a chromophore further comprises reducing the condensation product and cross-linking the reduced condensation product with the chromophore in one reaction step.

23. (New) The process as recited in claim 9 wherein the genetic material is bound to chromophore in aerobic conditions.

24. (New) The process as recited in claim 10 wherein the genetic material is bound to chromophore in anaerobic conditions.

25. (New) The process as recited in claim 8 wherein the two buffers comprise a first buffer to lyse the cells and a second buffer to attach the genetic material to the column.

If the 16S rRNA of any microorganism that did not belong to the genus of interest formed stable duplexes with any oligonucleotide considered as a potential probe for the microchip, this oligonucleotide was excluded from the list of probes. Oligonucleotides were synthesized with a 394 DNA/RNA Synthesizer (Perkin Elmer/Applied BioSystems, Foster City, CA) using standard phosphoramidite chemistry. 5¢-Amino-Modifier C6 (Glen Research, Sterling, VA) was linked to the 5¢-end of oligonucleotides. The microarray matrix containing 100´100´20-mm polyacrylamide gel pads fixed on a glass slide and spaced by 200 mm from each other was manufactured using photopolymerization D. Gushin, *Anal. Biochem.* **250**, 203-211, and incorporated herein by reference, and activated as described in D. Proudnikov et al. *Anal. Biochem.* **259**, 34-41, which is also incorporated herein by reference.

Predetermined aliquots of individual 1 mM amino-oligonucleotide solutions were applied to each gel pad, containing aldehyde groups. Schiff bases coupling the oligonucleotides with aldehyde groups within the gel pads were stabilized by reduction with $NaCNBH_3$ as described herein, and also in Timofeev et al. *Nucl. Acids Res.* **24**, 3142-3148 (1996), incorporated herein by reference.

Example 1
Hybridization of Total NA labeled
with OPCu using Oligo microarray

Up to 70 percent of total bacterial nucleic acid is rRNA. Therefore rRNA analysis is a common, sensitive and relatively simple method of bacterial identification.

FIG. 4 depicts the results when total nucleic acids are labeled with OP-Cu using the invented column device. FIG. 4A depicts the arrangement of the probes (having the sequences disclosed above) on the micro array. U1 and U2 represents "all life" (i.e., these probes screen for all procaryotic and eucaryotic cells except for some archibacteria). EU1 and EU2 represents all eubacteria. BSG1 and BSG2 represents *B. subtilis* group bacteria. BS1 and BS2 represents *B. subtilis* sp. BCG1 and BCG2 represents *cereus* group bacteria.

FIG. 4B depicts the analysis of *E. coli* hybridization with a stationary microscope.

FIG. 4C depicts the same analysis using a portable imager.

Normalized fluorescent signal intensities for labeled HL60 cells are depicted in FIG. 4D. Normalized intensities for *E. coli* are depicted in FIG. 4E. Signal intensities for *B. thuringiensis* and *B. subtilis* are depicted in FIGS. 4F and G, respectively. Fluorescent intensities were quantified using "Image", a custom LabView™ program available through National Instruments, Austin, TX.

Example 2
Hybridization of Total NA Labeled
with Fe-EDTA with Microarray

Example 2 provides data wherein a FeEDTA labeling method is used with the invented column protocol and device. Unlike the OP-Cu protocol, the FeEDTA method does not depend on the presence of reducing agents in the labeling cocktail.

Freshly prepared cocktail (135 ul) containing 30 ul of 5 mM EDTA/2.5mM ammonium iron (II) sulfate, 1 ul 100 mg/ml Lissamine rhodamine B ethylenediamine, 30 ul sodium phosphate (ph 7.0) and 74 ul DEPC-treated H_20 was preheated 95 °C for 30 sec. 15 ul of 100 mM H_2O_2 was added and immediately applied on the column containing total nucleic acids isolated from B. thuringiensis str. 4Q281. All other procedures were identical as in the OP-Cu protocol. Results of this hybridization is depicted in FIG. 5.

The microarray depicted in FIG. 5 is identical to the immobilized gel sequence arrangement depicted in FIG. 4. As can be noted, the entire first column of the array, from top to bottom (i.e., EU1, EU2, U1, and U2), is illuminated. Also, the first two cells in the third column from top to bottom (i.e., BSG1 and BSG2) are illuminated.

In summary, a procedure has been developed for nucleic acid isolation, labeling, and fragmentation within a single syringe-operated silica minicolumn. The process requires no vacuum filtration step, phenol-chloroform extraction, CsCl fractionation or centrifugation. This syringe-operated format is useful for field conditions. Alternatively, the syringe-based protocols can be replaced with centrifugation separations when the column protocol is utilized in laboratory settings. There are three main steps to the

Also provided is a two-buffer process for manipulating genetic material, the process comprising contacting cells containing the genetic material to a silica column; creating a first fraction of cell detritus and a second fraction containing the genetic material; confining the genetic material to the column; removing the cell detritus; subjecting the genetic material to radicals so as to produce reactive aldehyde groups on the genetic material; and attaching chromophore to the genetic material.

BRIEF DESCRIPTION OF THE DRAWING

The present invention together with the above and other objects and advantages may best be understood from the following detailed description of the embodiment of the invention illustrated in the drawing, wherein:

FIG. 1A is a schematic diagram of a column-based protocol for manipulating genetic material generally, in accordance with features of the present invention;

FIG. 1B is a schematic diagram of a column-based protocol for manipulating DNA and RNA solely, in accordance with features of the present invention;

FIG. 1 C is a diagram of a column positioned inferior to a column-pressurizing device, in accordance with features of the present invention;

FIG. 2A depicts oxidative cleavage of DNA [is a reaction sequence of DNA labeling], in accordance with features of the present invention;

FIG. 2B depicts a DNA intermediate in a labeling sequence, in accordance with features of the present invention;

FIG. 2C depicts a DNA-based aldehyde in a labeling sequence, in accordance with features of the present invention;

FIG. 3A depicts an RNA-based lactone in a labeling sequence , in accordance with features of the present invention;

FIG. 3B depicts an RNA-based cross-linking substrate used to form labeled product, in accordance with features of the present invention;

FIG. 4 A-G is a depiction of the hybridizations resulting from operation of the invented column process ; and

FIG. 5 is a diagram of a gel matrix depicting the efficiency of bacterial labeling utilizing features of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A column-based protocol for manipulating genetic material is provided herein. The column can be used alone or in combination with any microarray system. A salient feature of such a system include the invented column employed for successive DNA/RNA isolation, fractionation, fragmentation, fluorescent labeling, and removal of excess free label and short oligonucleotides. To demonstrate the efficiency of the column protocol the inventors used microarrays of immobilized oligonucleotide probes whereby the microarrays are juxtaposed at a depending end of the column; and a portable battery-powered device for imaging the hybridization of fluorescently labeled RNA fragments with the arrays.

The inventors have utilized the invented column in the above-identified configuration for 16S ribosomal RNA identification.

The inventors have exploited a phenomenon that nucleic acids bind to silica in the presence of high concentration of salt. To eliminate all centrifugation steps, heretofore required in typical protocols, a syringe column configuration can be utilized. As a result of this syringe configuration (depicted in FIG. 1C), the invented column-based protocol requires only two buffers. The isolation of total nucleic acids or the fractionation of DNA/RNA is effected in 3 to 5 minutes, as opposed to the typical 60-120 minute procedures employing four or more buffers, as discussed, supra. The buffers utilized are those effecting lysis and binding of the target genetic material.

The entire mini-column procedure, from cell lysis to removal of excess fluorescent label, is executed within 20-30 minutes.

The mini-column combines a method of nucleic acid isolation utilizing guanidine thiocynanate, with a newly developed hydroxyl radical-based technique for DNA/RNA labeling and fragmentation. The chemistry of nucleic acid isolation and DNA/RNA fractionation is effected via the application of the two buffer system, outlined infra.

If the 16S rRNA of any microorganism that did not belong to the genus of interest formed stable duplexes with any oligonucleotide considered as a potential probe for the microchip, this oligonucleotide was excluded from the list of probes. Oligonucleotides were synthesized with a 394 DNA/RNA Synthesizer (Perkin Elmer/Applied BioSystems, Foster City, CA) using standard phosphoramidite chemistry. 5¢-Amino-Modifier C6 (Glen Research, Sterling, VA) was linked to the 5¢-end of oligonucleotides. The microarray matrix containing 100´100´20-mm polyacrylamide gel pads fixed on a glass slide and spaced by 200 mm from each other was manufactured using photopolymerization D. Gushin, *Anal. Biochem.* **250**, 203-211, and incorporated herein by reference, and activated as described in D. Proudnikov et al. *Anal. Biochem.* **259**, 34-41, which is also incorporated herein by reference.

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